BTX-1188, a first-in-class dual degrader of GSPT1 and IKZF1/3, for treatment of acute myeloid leukemia (AML) and solid tumors

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BACKGROUND

- BTX-1188 is a first-in-class oral molecular glue that degrades GSPT1 (G1 to S phase transition 1 or eRF3a), a translation termination factor, and IKZF1/3 (Ikaros/Aiolos), transcription factors required for lymphocyte development and differentiation. It is currently in phase 1 clinical trials for treatment of hematologic and solid malignancies.
- Targeted protein degradation of Cereblon neosubstrates is clinically validated in the treatment of hematologic malignancies (Lu 2014, Zou 2020).

METHODS

- Cell viability in BTX-1188-treated cells and patient samples was measured by CellTiter-Glo 2.0 assay (Promega).
- Substrate degradation and apoptosis profiles were analyzed by immunoblots of protein lysates from cells treated with DMSO or BTX-1188.
- Human PBMCs were stimulated with aCD3 for IL-2 measurement or LPS-induced for measurement of inflammatory cytokines upon DMSO or compound treatment.
- Vehicle or BTX-1188 were used in athymic or BALB/c nude mice xenograft models.

RESULTS

- BTX-1188 is a rapid, deep, and potent degrader of GSPT1 and IKZF1/3 and inhibitor of MYC in several cancer cell lines.
- Proteomics and immunoblot analysis of AML cell line, MV-4-11, shows significant degradation of GSPT1 and IKZF1 after 2 hours treatment with 100 nM BTX-1188 ($P<1x10-5$) and 6 hours treatment with 3 nM BTX-1188 (>90% of GSPT1), respectively, indicating rapid and potent neosubstrate degradation.
- BTX-1188 also durably degrades GSPT1 where treatment with 30 nM for 6 hours followed by washout maintains significantly lower levels of GSPT1 and sustained apoptosis as seen by PARP cleavage for up to 24 hours. This durability of GSPT1 degradation by BTX-1188 functionally manifests as low nanomolar IC_{50} values despite 72-hour of compound washout.
- Owing to IKZF1/3 degradation, BTX-1188 has immunomodulatory properties as seen by inhibition of proinflammatory cytokines (IL-1b, IL-6, TNF-a) and induction of IL-2 by LPS and aCD3-stimulated PBMCs, respectively. This approach is expected to improve clinical outcomes and reduce toxicities associated with selective GSPT1 degradation (CC-90009), thus expanding the therapeutic window of BTX-1188.
- Functionally, BTX-1188 is cytotoxic in various cancer cell lines such as MYC-dependent and non-MYC dependent lines (IC_{50} range: 0.5-20 nM) and primary human AML patient samples (IC_{50} range: 0.4-1.5 nM), including relapsed/refractory-, cytarabine- and Venetoclax-resistant samples.
- The durability of GSPT1 degradation and sustained apoptosis in response to BTX-1188 treatment is further reflected in *in vivo* efficacy models where daily or intermittent dosing of BTX-1188 results in significant and sustained antitumor activity.

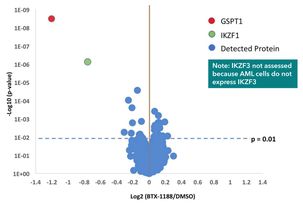
BTX-1188 is a rapid and deep degrader of GSPT1 and IKZF1/3

DC_{50} and IC_{50} of BTX-1188 at 6 hours of treatment

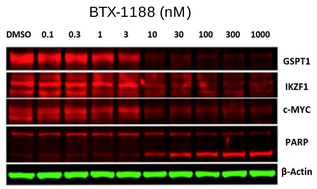
BTX-1188 treated cancer cell lines	DC_{50} (nM)			IC_{50} (nM)	
	GSPT1	IKZF1	IKZF3	c-MYC	n-MYC
NCI-H1155 (NSCLC)	2	–	–	NI	10-30
ABC-1 (NSCLC)	2	–	–	NI	11
DOHH-2 (lymphoma)	0.2	1	1	1	–
SU-DHL-2 (DLBCL)	3	–	–	28	–
Daudi (Burkitt lymphoma)	2	–	–	12-15	–
KNS-42 (glioma)	4	–	–	NI	3

DC_{50}, degradation concentration; DLBCL, diffuse large B cell lymphoma; NI, no impact; NSCLC, non-small cell lung cancer

Proteomics and immunoblot analysis of BTX-1188 in AML



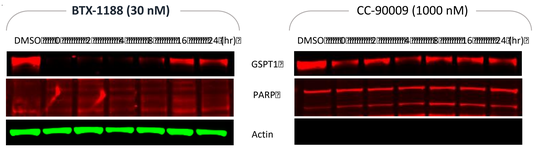
Volcano plot of tandem mass tag proteomics analysis of MV-4-11 (AML) cells treated with BTX-1188 (100 nM) for 2 hours



Immunoblot of MV-4-11 cells treated with compounds for 6 hours

BTX-1188 is a durable degrader of GSPT1

Durability of GSPT1 degradation upon washout



Immunoblot of MV-4-11 cells treated with compounds for 6 hours followed by washout and lysate collection at 2-, 4-, 8-, 16- and 24-hours post-washout

Durability of GSPT1 degradation impacts cell viability

IC_{50} (nM) in AML cell lines	BTX-1188	CC-90009
Molm-13 (No Washout)	1.5	166
MV-4-11 (No Washout)	0.9	24
Molm-13 (With Washout)	20	4434
MV-4-11 (With Washout)	9	7415

IC_{50} of cells without washout or with washout (treated with compounds for 8 hours followed by cell viability measurement at 72-hours post-washout)

BTX-1188 enhances immune stimulatory cytokines and inhibits pro-inflammatory cytokines

Immune cytokine induction (fold increase vs. baseline of 1.0)

IL-2 Induction	BTX-1188	CC-90009	Pomalidomide
1 nM	2.1x	1.3x	1.3x
0.1 nM	1.7x	1.2x	1.0x

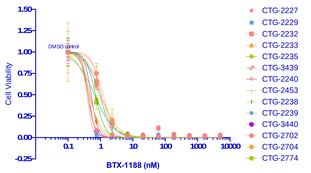
Inflammatory cytokine inhibition

IC_{50} (nM)	BTX-1188	CC-90009	Pomalidomide
IL-1b	2.1	>1000	14.5
IL-6	4.5	>1000	673.6
TNF-a	2.9	>1000	14.8

αCD3-stimulated and LPS-induced cytokine induction in human PBMCs

BTX-1188 potently inhibits AML and solid tumor cell proliferation

BTX-1188 is highly active in primary human AML patient samples



Samples included:
- Relapsed/refractory disease state (6/15 samples)
- Cytarabine- and Venetoclax-resistant (4/15 samples)

BTX-1188 is highly active in AML cell lines compared to CC-90009

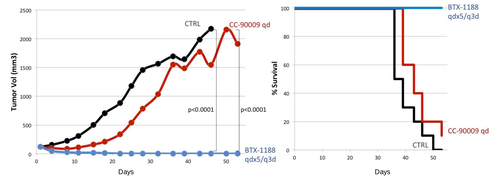
Human AML cell lines	IC_{50} (nM) of compounds	
	BTX-1188	CC-90009
Molm-13	1.5	157
MV-4-11	0.9	29
KG-1	4	177
HL-60	1.1	28
Kasumi-1	1.2	18
AML-193	0.5	12

BTX-1188 inhibits solid tumor cell lines

Human Cancer Cell Lines	Tissue	BTX-1188 IC50 (uM)
BT-549	Breast	0.042
HCC1937	Breast	0.054
SK-Br-3	Breast	0.022
T47D	Breast	0.007
NCI-H889	Lung	0.048
HCC827	Lung	0.036
NCI-H82	Lung	0.026
Calu-6	Lung	0.016
NCI-H1155	Lung	0.002
ABC-1	Lung	0.002
A2780	Ovary	0.007
IGROV-1	Ovary	0.011
OVCAR-4	Ovary	0.079
OVCAR-8	Ovary	0.01

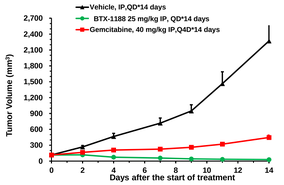
BTX-1188 inhibits AML tumor growth

BTX-1188 is highly efficacious in MV-4-11 xenograft in athymic nude mice

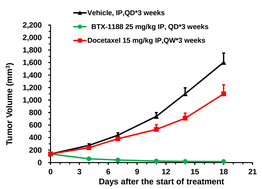


BTX-1188 inhibits solid tumor growth

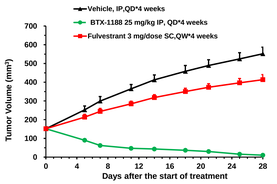
BTX-1188 is highly efficacious in NCI-H1155 (NSCLC) xenograft model in BALB/c nude mice



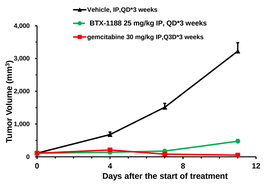
BTX-1188 is highly efficacious in NCI-H526 (SCLC) xenograft model in BALB/c nude mice



BTX-1188 is highly efficacious in T47D (Breast cancer) xenograft model in BALB/c nude mice



BTX-1188 is highly efficacious in A2780 (Ovarian cancer) xenograft model in BALB/c nude mice



CONCLUSIONS

These preclinical data show that BTX-1188 is a promising drug candidate for AML and solid tumors with greater sensitivity in MYC dependent tumors. Its immunomodulatory properties owing to IKZF1/3 degradation may prevent systemic inflammatory dose-limiting toxicities associated with pure GSPT1 degradation (Uy 2019). BTX-1188 has entered phase 1 clinical studies for advanced solid tumors and AML.

REFERENCES

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3. Uy, G.L., et al. *Blood* **134** (Supplement_1): 232 (2019)

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